Retinal Organoids Long-Term Functional Characterization Using Two-Photon Fluorescence Lifetime and Hyperspectral Microscopy.

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Public Summary:
Pluripotent stem cell-derived organoid technologies have opened avenues to preclinical basic science research, drug discovery, and transplantation therapy in organ systems. However, heterogeneous tissue yields and subjective tissue selection reduce the repeatability of organoid-based scientific experiments and clinical studies. To improve the quality control of retinal organoids, we introduced a live imaging technique based on two-photon microscopy to noninvasively monitor and characterize retinal organoids' long-term development. Fluorescence lifetime microscopy was used for the monitoring of the metabolic trajectory, and hyperspectral imaging was applied for the characterization of structural and molecular changes. We found that the metabolic activity ascertained from the free-to-bound NADH ratio shifted from a more glycolytic and proliferative stem cell phase to more oxidative phosphorylation consistent with differentiated phase with stabilized glycolytic photoreceptor layer during maturation. The data showed a consistent developmental trend of retinal organoids across different cell lines and different manufacturing batches. Results were further confirmed by molecular analysis and immunohistology. The methodology and the findings of this study are of great importance in live retinal organoids characterization and monitoring, as well as screening and quality control for further applications including transplantation therapy for retinal degenerative diseases.

Scientific Abstract:
Pluripotent stem cell-derived organoid technologies have opened avenues to preclinical basic science research, drug discovery, and transplantation therapy in organ systems. Stem cell-derived organoids follow a time course similar to species-specific organ gestation in vivo. However, heterogeneous tissue yields, and subjective tissue selection reduce the repeatability of organoid-based scientific experiments and clinical studies. To improve the quality control of organoids, we introduced a live imaging technique based on two-photon microscopy to non-invasively monitor and characterize retinal organoids' (RtOgs') long-term development. Fluorescence lifetime imaging microscopy (FLIM) was used to monitor the metabolic trajectory, and hyperspectral imaging was applied to characterize structural and molecular changes. We further validated the live imaging experimental results with endpoint biological tests, including quantitative polymerase chain reaction (qPCR), single-cell RNA sequencing, and immunohistochemistry. With FLIM results, we analyzed the free/bound nicotinamide adenine dinucleotide (f/b NADH) ratio of the imaged regions and found that there was a metabolic shift from glycolysis to oxidative phosphorylation. This shift occurred between the second and third months of differentiation. The total metabolic activity shifted slightly back toward glycolysis between the third and fourth months and stayed relatively stable between the fourth and sixth months. Consistency in organoid development among cell lines and production lots was examined. Molecular analysis showed that retinal progenitor genes were expressed in all groups between days 51 and 159. Photoreceptor gene expression emerged around the second month of differentiation, which corresponded to the shift in the f/b NADH ratio. RtOgs between 3 and 6 months of differentiation exhibited photoreceptor gene expression levels that were between the native human fetal and adult retina gene expression levels. The occurrence of cone opsin expression (OPN1 SW and OPN1 LW) indicated the maturation of photoreceptors in the fourth month of differentiation, which was consistent with the stabilized level of f/b NADH ratio starting from 4 months. Endpoint single-cell RNA and immunohistology data showed that the cellular compositions and lamination of RtOgs at different developmental stages followed those in vivo.